Urobilinogen Excretion in Normal Adults
Results of Assays with Notes on Methodology

Bernard Balikov

The quantitative estimation of urobilinogen in feces and urine in the evaluation of hepatic and hemolytic diseases has become widely accepted as a useful tool both for clinical and research purposes. Our interest in urobilinogen assays stemmed principally from a study of hepatic function subsequent to major thermal trauma. Of the several methods available for this analysis, the one based on the works of Watson and his associates is probably most widely used, and was the one employed in this study. However, several significant changes in methodology have taken place since Watson first published his normal ranges in 1937 (1). Consequently, it was felt that a reassessment of these values would improve the reliability of any interpretations we made of our findings.

This report principally concerns the results of assays on 46 adults for urine urobilinogen and 42 for fecal urobilinogen utilized so as to reveal statistically valid information concerning normal values for this modified procedure.

Material and Methods

Subjects were assumed to be normal if they had no illness at the time samples were collected, and had no history of jaundice, gastrointestinal ulcers, malaria, or blood dyscrasias. Samples were not collected on

---

From the Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas.
Present address: Department of Metabolism, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C.
Received for publication July 1, 1956.
females during menstrual periods. Subjects were between 18 and 61 years of age and were divided into four groups, which will hereafter be referred to by their group letters: Group A, males weighing 70 kg. and less; Group B, males weighing more than 70 kg.; Group C, females weighing less than 56 kg.; and Group D, females weighing 56 kg. and more.

Urobilinogen in both urine and feces was assayed by the quantitative methods of Schwartz, Sborov, and Watson (2) as modified by Balikov (3). The standard curve using pontacyl dyes was used as described by Watson and Hawkinson (4), and readings were made at 565 m\(\mu\) on the Coleman Junior spectrophotometer, Model 6A, using 19 x 150 mm. cuvets. It should be noted that these methods involve reduction with Fe(OH)\(\text{2}\) and subsequent extraction with petroleum benz in as differentiated from the "rapid" methods which do not include either or both of these steps.

Urine assays were all made on aliquots of 24-hour collections. Samples were collected in painted or paper-covered jugs which contained 5 Gm. of anhydrous Na\(_2\)CO\(_3\) and 100 ml. of petroleum benz in. All analyses were completed the day the collection was finished.

Feces were collected over a 4-to-5-day period in weighed, waxed containers with airtight lids. Samples were stored in a refrigerator until the collection was complete. Aliquots were taken from a blend made by mixing all samples collected during a single period in a Waring Blender with measured amounts of water sufficient for easy stirring. Assays were usually completed the day the last sample of a series was collected. In no case were analyses postponed longer than one more day.

Notes on the Method

The literature frequently refers to the use of "saturated sodium acetate" without specifying whether to use the anhydrous or triple-hydrated salt. Since the function of the acetate is to convert the free HCl to acetic acid (5) it was felt that perhaps either may be used. To check this possibility, saturated solutions were made of both salts by simply adding an excess to water, so that some undissolved solid was always present. The two acetate solutions were used on identical aliquots of the same ferrous hydroxide filtrates. Two urine and 4 fecal samples showed no determinate difference between the two solutions (Table 1). Thus, the less expensive triple-hydrated salt prepared as indicated above was used exclusively in this work.

It has been reported that the urobilinogen-aldehyde color fades on standing and that the stability of this color depends to some extent on the purity and amount of sodium acetate used (2). Using only reagent
grade triple-hydrated sodium acetate in a 3:1 ratio to the Ehrlich’s reagent, this color was found to be stable for at least 70 minutes both in the dark and in ordinary artificial light (Table 2).

A check was made for the completeness of the extraction of urobilinogen with petroleum benzin from the acidified Fe(OH)₂ filtrate. After several trials in which the number and time of extractions were varied it was found that using 35 ml. of petroleum benzin, a single, vigorous extraction for 7 minutes would completely extract the urobilinogen. A second extraction made on several normal and pathologic urine and fecal specimens consistently showed 99 per cent transmittance or more. However, it is strongly recommended that the individual actually performing this assay make a similar check for completeness of extraction.

It has been found to be of utmost importance to complete an extraction with the modified Ehrlich’s reagent before the sodium acetate is added. If the two reagents are added simultaneously, results will be from 50 to 75 per cent lower.
RESULTS

Urine

Using the t test for significance (6), Groups A, B, C, and D were compared with each other in an attempt to determine if there were any significant differences between their means (Table 3). Since all P values are greater than 0.05, all four groups may be considered parts of the same population. However, the mean of Group A (1.2) is twice as high as that of the next highest group (Group B = 0.6), which indicates an unexplained tendency for higher values in lighter-weight males.

<table>
<thead>
<tr>
<th></th>
<th>A vs. B</th>
<th>A vs. C</th>
<th>A vs. D</th>
<th>B vs. C</th>
<th>B vs. D</th>
<th>C vs. D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>0.6</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>No. of samples</td>
<td>14</td>
<td>10</td>
<td>14</td>
<td>11</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Combined</td>
<td>1.06</td>
<td>0.93</td>
<td>0.97</td>
<td>0.34</td>
<td>0.45</td>
<td>0.49</td>
</tr>
<tr>
<td>SD</td>
<td>1.27</td>
<td>1.96</td>
<td>1.65</td>
<td>0.79</td>
<td>0.30</td>
<td>0.33</td>
</tr>
<tr>
<td>t</td>
<td>0.2-0.3</td>
<td>0.1-0.05</td>
<td>0.1-0.2</td>
<td>0.4-0.5</td>
<td>0.7-0.8</td>
<td>0.7-0.8</td>
</tr>
</tbody>
</table>


The 46 urine urobilinogen values were checked for normalcy of distribution by a plot against the Normal Equivalent Deviate values according to the method of Moore and associates (7). By inspection, the distribution did not appear normal, but a plot of the logarithms of these urobilinogen values does so appear (Fig. 1). Thus, these logarithm values were used in calculating the normal range. The pertinent results are as follows:

\[
\text{Mean} = 0.45
\]

\[
\text{Standard deviation (SD)} = \pm 0.425
\]

\[
\text{Normal range (mean } \pm 2\text{SD)} = -1.30 \text{ to } 0.40
\]

Conversion of these logarithms to their antilogarithms yields the following results:

\[
\text{Mean (more correctly, Median)} = 0.36 \text{ mg./24 hr.}
\]

\[
\text{Normal range} = 0.05 \text{ to } 2.5 \text{ mg./24 hr.}
\]
UROBILINOGEN EXCRETION IN NORMAL ADULTS

Fig. 1. Lognormal distribution of urine urobilinogen values on adults. The dots represent values in terms of milligrams/24 hours, the circles represent the logarithms of these values. The lines are drawn to emphasize the curvature of the one set of points compared with the relative linearity of the other.

Feces

As for urine, the $t$ test for significance was used to compare the fecal excretion of urobilinogen of the four groups (Table 4). Although differences significant at the 1% level are found between three of the pairs (A vs. C, B vs. C, and B vs. D), no significant differences are found between the male groups (A and B) or the female groups (C and D). There-
TABLE 4. SIGNIFICANCE OF THE DIFFERENCES OF THE MEANS OF FECAL UROBILINOGEN EXCRETION BETWEEN VARIOUS POPULATION GROUPS

<table>
<thead>
<tr>
<th></th>
<th>A vs. B</th>
<th>A vs. C</th>
<th>A vs. D</th>
<th>B vs. C</th>
<th>B vs. D</th>
<th>C vs. D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>103.122</td>
<td>103.37</td>
<td>103.70</td>
<td>122.37</td>
<td>122.70</td>
<td>37.70</td>
</tr>
<tr>
<td>No. of samples</td>
<td>10</td>
<td>11</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Combined SD</td>
<td>34.6</td>
<td>28.9</td>
<td>45.5</td>
<td>28.5</td>
<td>43.0</td>
<td>38.1</td>
</tr>
<tr>
<td>t</td>
<td>1.26</td>
<td>5.23</td>
<td>1.62</td>
<td>7.54</td>
<td>2.77</td>
<td>1.98</td>
</tr>
<tr>
<td>P*</td>
<td>0.2-0.3</td>
<td>0.01</td>
<td>0.1-0.2</td>
<td>0.01</td>
<td>0.01-0.02</td>
<td>0.05-0.10</td>
</tr>
</tbody>
</table>


Fig. 2. Lognormal distribution of fecal urobilinogen values on adult males. The dots represent values in terms of milligrams/24 hours, the circles represent the logarithms of these values. The lines are drawn to emphasize the curvature of the one set of points compared with the relative linearity of the other.
fore, all males were combined and compared with all females. These groups were found to be different at a level greater than 99 per cent ($P < .01$).

The normalcy of distribution of the values for each of the two groups (adult males and adult females) was checked as for urine urobilinogen above. It is apparent from Figs. 2 and 3 that the logarithms of the values for both groups are more normally distributed than the values themselves. As for urines, then, the logarithms were used in calculating the normal ranges. The pertinent results for males are:

$$\text{Mean} = 2.032$$
$$\text{SD} = \pm0.138$$
$$\text{Normal range} = 1.756 \text{ to } 2.308$$
Conversion of the logarithms to their antilogarithms yields:

\[
\text{Median} = 101 \text{ mg./24 hr.} \\
\text{Normal range} = 57 \text{ to } 200 \text{ mg./24 hr.}
\]

The results on adult females are:

\[
\text{Mean} = 1.608 \\
\text{SD} = \pm 0.328 \\
\text{Normal range} = 0.952 \text{ to } 2.264
\]

Conversion of the logarithms to their antilogarithms yields:

\[
\text{Median} = 40 \text{ mg./24 hrs.} \\
\text{Normal range} = 8 \text{ to } 150 \text{ mg./24 hr.}
\]

**DISCUSSION**

The grouping of subjects by sex, age, and weight was based on the following considerations. The urobilinogen found in urine and feces is formed at least in large part from bile which in turn is formed from hemoglobin (8). Since the blood hemoglobin concentration varies with age (up to 16 years) and sex (9) and the blood volume varies with weight, it was felt that these factors may be variables in the production and consequent elimination of urobilinogen. Our subjects were all over 18 and the values reported here should not be applied to children without further investigation. The mean weights of 70 kg. for men and 56 kg. for women were taken from data reported in Sunderman and Boerner (10).

There is no apparent explanation for the logarithmic distribution of normal values. This type of distribution has been noted for a number of blood constituents including bilirubin (11) and so perhaps should not be surprising when found in excretory pigments as well.

Since it has been shown that apparent minor changes in method can result in significant differences in results (3), the normal values reported here should not be applied to results obtained using any but the described procedure, unless further investigation proves such an application to be valid.

**SUMMARY**

Using the quantitative methods of Schwartz, Sborov, and Watson, as modified by Balikov, normal ranges for excretion of urobilinogen have been determined for adults. The individual values fall into a logarithmic type of distribution.
The normal range for urine urobinogen excretion is from 0.05 to 2.5 mg./24 hours, with a median of 0.36. The excretion of fecal urobinogen ranges from 57 to 200 mg./24 hours for males and from 8 to 150 mg. for females. The medians for these groups are 101 and 40 mg., respectively.

REFERENCES